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RESEARCH **Open Access**

The Assay of Blood Plasma's Malondialdehyde (MDA) Activity in Alloxan-Induced Diabetic Rat Given Yellow Velvet Leaf Extract (*Limnocharis flava)*

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Abstract

It has been well-known that Genjer or yellow velvet leaf (*Limnocharis flava*) has a potential as an alternative source to natural antioxidants. Antioxidants, such as flavonoids, alkaloids, phenols, tannins, and others are substances which can block the presence of free radicals coming into the body. It has also been examined to be beneficial in treating metabolic syndrome diseases such as diabetes, as it improves insulin's performance. The objective of this study is to examine the antioxidant effect of yellow velvet leaf's ethanolic extract (*Limnocharis Flava*) on blood plasma's MDA activity in alloxan-induced diabetic rats (*Rattus novergicus*) in three different doses. The testing animals were randomly divided into five groups, each group consisted of 6 white rats. Group 1 was the negative control and given CMC Na 0.5%, while group 2 was a positive control and given glibenclamide 0.45 mg, group 3 was yellow velvet leaf's ethanolic extract of 32.5 mg/kg, group 4 was yellow velvet leaf's ethanolic extract of 65 mg/kg, group 5 was yellow velvet leaf's ethanolic extract of 130 mg/kg. Previously, all groups were induced with alloxan through intra peritoneal injection. Ethanol extract was provided once on the day of 7th, 14th, and 21st. The observed parameters were blood plasma's MDA activity in the alloxan-induced diabetic rats (*Rattus novergicus*). Blood plasma's MDA activities were assessed by using Thiobarbituric Acid Reactive Substance (TBARs) method. Then, data were collected and analyzed by using One Way ANOVA followed by a Post hoc test. The results showed that the mean values of MDA levels in the testing groups of 1,2,3,4 and 5 were 9.30 ± 0.462, 2.17 ± 0.121, 6.11 ± 0.381, 4.07 ± 0.327, and 2.75 ± 0.121, respectively. One Way ANOVA test showed a significant difference in the blood plasma's MDA levels among the groups (p = 0,000). It is concluded that the blood plasma's MDA levels in alloxan-induced diabetic rats can be lowered by the yellow velvet leaf's ethanolic extract (*Limnocharis Flava*). Therefore, it can be employed as a traditional treatment for diabetes.

Keywords: Blood plasma's MDA, Yellow velvet leaf, Antioxidants

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1. INTRODUCTION

Diabetes Mellitus is a group of metabolic diseases with hyperglycemia characteristic which occurs due to the abnormalities in insulin secretion, insulin action, or both disorders (Aprilia, et al., 2018). The condition of hyperglycemia in diabetes mellitus has a tremendous effect on the endothelium of blood vessels due to the glucose auto-oxidation process in forming free radicals, which result in macro and microvascular dysfunction (Monroy, et al, 2013). This condition can cause complications which increase the morbidity and mortality rates in people with diabetes mellitus. The use of antioxidants in diabetes mellitus sufferers is effective in reducing the occurrence of complications. It is supported by various studies which prove the benefits of antioxidants related to the pathological process of diabetes mellitus due to the oxidative stress conditions (Prawitasari, 2019).

High blood glucose levels may trigger an increase in glucose autoxidation and in protein glycation (Khangholi, et al., 2016). The bonding of AGEs with their receptors on the cell membrane influences the formation of free radicals. The number of which exceeds the number of antioxidants in the body and may affect oxidative stress (Nowotny, et al, 2015).

Free radicals cause oxidative stress. Oxidative stress occurs because of an imbalance between oxidants and antioxidants, which then causes cell and liver damage (Elgaml & Hashish, 2014). Free radicals can also increase the lipid peroxidation which decomposes into malondialdehyde (MDA) in the blood. MDA is a marker of cellular damage from free radicals (Zaetun, 2019). On the other hand, the occurrence of excess free fatty acids or hyperlipidemic conditions can cause overproduction of ROS, which in turn results in DNA mitochondria damage and malfunctions of the pancreatic cells which will all have an impact on the emergence of oxidative stress in diabetes (Oyenihi, et al., 2015).

Antioxidants are active compounds which can ward off free radicals. Antioxidants possess the ability to neutralize free radicals from their original form (Widyawati, et al., 2016). Flavonoids are polyphenolic compounds which inhibit the formation of AGEs and oxidation reactions by donating one electron to an unpaired electron to free radicals, thus, reducing the number of free radicals (Khangholi, 2016).

In Narwanti & Hamida, (2018) research, it was revealed that ethanol extract has a greater potential as a free radical destroyer comparing to the n-hexane fraction, chloroform fraction, and ethyl acetate fraction. Furthermore, in Nurjanah's, et al., research (2014), the results of the crude extract of fresh yellow velvet which underwent steaming, has antioxidant activity. Fresh yellow velvet extract has the tallest antioxidants.

Genjeror yellow velvet is a type of water plant spread throughout the mainland Asia and originated from America. Genjer is used by the community as food which is able to increase appetite and improve digestion. Yellow velvetleaf is a weed plant growing in the watery environments. Yellow velvetleaf is frequently used by the community as a vegetable. A group of compounds contained in simplicia, genjer plant ethanol extract is a group of tannin compounds, quinones, polyphenols, flavonoids, and steroids/terpenoids (Prasadhana, 2019). Based on the previous studies, it is revealed that yellow velvet leaves contain antioxidants in the form of flavonoids. Further research is needed to see the effect of ethanol extract of yellow velvet leaves (*Limnocharis Flava*) on the activity of MDA (*Malondialdehyde*) blood plasma in alloxan-induced diabetic rats.

2. RESEARCH METHOD

The statistical method employed in this study was One Way ANOVA, which aims to evaluate the differences between group representatives, after which it was followed by the Post-hoc test. In examining MDA blood plasma activity, the Thiobarbituric Acid Reactive Substance (TBARs) method was administered.

The tools used in this study were a syringe (Terumo), mortar and stamper, measuring flask (pyrex), digital balance (mettle toledo), rotary evaporator, blender (cosmos), measuring cup (Pyrex), beaker glass (pyrex), rod mixer (pyrex), silica gel GF 254, test tube (pyrex), oven (cosmos), chamber, capillary tube, separating funnel (pyrex), filter paper, scissors, 40 mesh sieve, water bath, mouse cage, Thiobarbituric Acid Reactive Substance (TBARs) assay kit, 759 S UV-vis Spectrophotometer, and moisture content measuring device. The materials were fresh yellow velvet leaf's (*Limnocharis Flava*) leaves, 70% ethanol solvent, CMC Na (negative control), glibenclamide (positive control), Vaseline album base, and TLC mobile phase.

Samples identified as Genjer or yellow velvetleaf (*Limnocharis Flava*) in the central laboratory of STIFAR "Yayasan Farmasi" (Certificate Number 1353/15-LBF/STIFAR/S.Ket-Det/V/2019), Semarang, Central Java, were chopped, dried, and mashed. Then, the moisture content was measured. Furthermore, as much as 1500 mg of powder, which has been sifted by using a 40-mesh sieve, was macerated with 70% ethanol for three days, accompanied by stirring every day. The macerate was then concentrated by using a water bath to produce a thick extract. The extracts were divided into three variations of the dosage, which were 32.5 mg/kg, 65 mg/kg, and 130 mg/kg. The test animals were induced with alloxan through intraperitoneal injection. Then, the appropriate treatments were applied to each group on days 7, 14, and 21. The test animals were sacrificed, and the blood was drawn. The blood samples were centrifuged at 2500 rpm for 8 minutes. The separating serum was piped using a micropipette and placed into the effendorf tube. A total of 0.5 ml of serum was administered to the test tube and added by 1.25 ml of 40% TCA, 0.2 ml of 1 NHCl, 0.5 ml of aquabides, and 0.1 ml of Na-Thiosulphate. Then, the mixture was heated at 1000oC for 25 minutes using a supernatant heating machine. The liquid was centrifuged for 5 minutes in 3000 rpm. The supernatant formed was obtained and placed in the vacuum tube, and the aquabides was added into the supernatant up to 3 ml. The supernatant was inserted into a 759 S UV-VIS Spectrophotometer, while the absorbance was measured at a wavelength of 523. This research has received ethical permission from the Medical / Health Research Bioethics Commission, Faculty of Health, Sultan Agung Islamic University Semarang No. 298 / IX / 2020 / Bioethical Commission.

3. RESULTS AND DISCUSSION

Oxidative stress can be monitored using Thiobarbituric Acid Reactive Substances (TBARS) test using Malondialdehyde (MDA) level as a marker. Thiobarbituric Acid Reactive Substances are the most frequently used test to measure the oxidative stress levels and the results of the lipid oxidation, which is MDA**.**

Blood plasma MDA level data from all tested animals were statistically analyzed by one-way ANOVA to examine the differences between the treatment groups. The results of the analysis test are presented in table 1 below:

Table 1. Comparison of blood plasma MDA levels in alloxan-induced diabetic rats between test groups

Groups	N	mean blood plasma MDA levels ± SD
		MDA (nmol / ml)
K (-)	6	9,3± 0,46
K (+)	6	2,17± 0,12
P1	6	6,11± 0,38
P2	6	4,07± 0,32
P3	6	2,75± 0,12

The value shows that the mean SD ± in each group is significant (p-value <0.05)
a = Extract treatment groups 1,2 and 3 were compared with the positive control group
ns = Non Signifikan

Based on the results of the analysis as shown in Table 1, MDA levels of blood plasma in alloxan-induced diabetic rats given ethanol extract of genjer/yellow velvet leaf were significantly different ($p<0.05$) with the negative control group. It implies that the ethanol extract of yellow velvet leaf has high antioxidant activity, thus it is able to reduce the blood plasma MDA levels. Moreover, when compared with positive controls, there was no significant difference ($p>0.05$) from the blood plasma MDA level of the three doses of yellow velvet leaf ethanol extract groups. It indicates that the ethanol extract of yellow velvet leaf is able to reduce MDA level in blood plasma as good as those shown by glibenclamide.

The decrease in MDA blood plasma levels in diabetic rats was caused by the presence of antioxidants in yellow velvet leaf samples, and it had also been evidenced through the phytochemical screenings that yellow velvet leaf contained flavonoids, alkaloids, and tannins. Zakaria, et al. (2011) explained that non-nutrient compounds such as polyphenols, flavonoids, and phenols function as antioxidants. Furthermore, Bajaj & Khan, (2012) added that antioxidants coming from outside the body obtained from vegetables and fruits can replace the low levels of antioxidants in the body, which are frequently discovered in diabetes.

The proven antioxidant provision may lower MDA levels. Antioxidants can also reduce MDA in a direct and indirect way, which is by means of capturing ROS directly, and an indirect mechanism by inducing the antioxidant enzymes, inhibiting prooxidant enzymes, and producing phase detoxification enzymes II and antioxidant enzymes (Situmorang & Zulham, 2020).

To determine the effect or antioxidant activity of yellow velvet leaf, Alloxan was employed to damage pancreatic β cells, which cause diabetes. Alloxan is an unstable hydrophilic compound and is selectively toxic to the liver and kidneys. However, at certain doses, it causes selective damage to pancreatic beta cells. Pure Alloxan was obtained from the oxidation of uric acid by nitric acid. The half-life of Alloxan at a pH of 7.4 and a temperature of 37oC was1.5 minutes and is very easily oxidized (Yuda, et al., 2013).

Flavonoids can act as antioxidants because they own a hydroxyl group attached to an aromatic ring, thus, they capture free radicals resulting from oxidation of lipids and glucose (Astuti, 2012). Flavonoids act as a hydrogen transfer to stabilize free radical molecules. Moreover, Rajendiran, et al. (2018) in a review on the role of antioxidants in diabetes argued that bioactive compounds such as polysaccharides, sterols, triterpenoids, alkaloids, flavonoids, fats, coumarins, phenols, and peptides have roles as antioxidants which restore the pancreatic β cell's function.

Widyaningsih's, et al, research (2016) shows that the ethanol extract administration green algae (Ulva Lactuca L.) acquire antioxidants activity with parameters decreased levels of MDA and increased hepatic SOD activity of CCl4-induced mice. From this study, it was also proved that the antioxidant content contained in plants can reduce MDA levels.

For additional knowledge about the benefits of genjer or yellow velvet leaves, in a study conducted by Juhaeti, (2013), the results obtained that genjer contains crude fiber (1.56%,1.42%), protein (2.04% and 1.98%), and carbohydrates (3.16% and 2.98%) in which the values were quite high compared to caisin and spinach. Genjer's leaf and flower contain 9 types of essential amino acids and eight kinds of non-essential amino acids.

4. CONCLUSION

Based on the results of the research, it can be concluded that the ethanol extract of yellow velvet leaf (*Limnocharis Flava* L) can reduce the blood plasma MDA levels of alloxan-induced diabetic rats. Hence, it has an antioxidant effect. The dose of ethanol extract, which is the most effective as an antioxidant, is 130 mg/kg BW because it shows the lowest blood plasma MDA level.

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